Resource-Efficient Technologies 3 (2017) 137–143

Contents lists available at ScienceDirect

Resource-Efficient Technologies

journal homepage: www.elsevier.com/locate/reffit

Quality-by-Design (QbD) process evaluation for phytopharmaceuticals on the example of 10-deacetylbaccatin III from yew ☆

Lukas Uhlenbrock, Maximilian Sixt, Jochen Strube*

Institute for Separation and Process Technology, Clausthal University of Technology, Clausthal-Zellerfeld 38678, Germany

ARTICLE INFO

Article history:
Received 21 December 2016
Revised 9 February 2017
Accepted 1 March 2017
Available online 18 April 2017

Keywords:
Quality by Design
Yew
Process design
Chromatography

ABSTRACT

The focus of pharmaceutical product development lies on assuring excellent product quality at the end of a cost-efficient process. The Quality-by-Design (QbD) concept shifts the focus from quality assurance through testing to quality control by process understanding, resulting in very robust processes with high quality product. QbD was originally intended by authorities for biologics, where product quality proven completely by analytics is not desired. Product quality has to be controlled by means of appropriate processes and operations as well.

These demands were developed in order to improve patients' safety by optimal drug quality at more efficient manufacturing processes reducing costs for healthcare systems. Furthermore, production of biologics includes feedstock variability and complex multi-step manufacturing processes in batch operation with variable lots – condition, which apply to botanicals as well.

The use of rigorous (physico-chemical) process modeling in combination with QbD results in a high degree of process understanding. This offers, contrary to popular prejudices, great benefit for manufacturers with little extra effort during development.

The methodical QbD-based approach is pursued to develop a process for extraction and purification of 10-deacetylbaccatin III from yew needles. A short history and key elements of the QbD-based application are introduced.

The line of argument for basic process conception is described and initial risk assessment is shown. Typical raw material variation and vaporization are identified as causes of process variability, therefore, the implications to subsequent process steps are pointed out. Finally, influences of load and flow rate on the chromatographic separation of 10-deacetylbaccatin III are shown to exemplify sensitivity of purification design.

1. Introduction

The positive effects of plants on human health have been used for centuries and are well accepted by patients to the present day. The efficacy of **phytotherapeutics** often results from a complex composition of substances [1,2]. The complexity of plant-based drugs is one reason for the lack of satisfactory studies into their effect and causes problems for the definition and assurance of quality [3]. Although "good manufacturing practice" (GMP) is acknowledged as an important tool in quality assurance, the regulatory approach for most problems varies between the competent agencies (e.g. FDA, EMA) [4,5], which are responsible for the scientific evaluation of the market applications. In addition, more political support is needed to improve integration of traditional and complementary medicine into health systems [6]. The World Health Organization (WHO) recognizes these challenges and proposes a new strategy regulating traditional herbal medicines. [7].

Quality assurance is realized by the implementation of GMP and is well established in the industry for all types of medicines [8]. The Food and Drug Administration (FDA) reviewed the regulatory approach to GMP practices at that time and introduced a modernized version with the focus on process risk determination, risk mitigation, and risk control to promote the development of robust and efficient processes [9]. This approach has been adopted and further developed by the European Medicines Agency (EMA) and other communities and culminates in the **"Quality by Design"**

☆ Peer review under responsibility of Tomsk Polytechnic University.

* Corresponding author. Institute for Separation and Process Technology, Clausthal University of Technology, Clausthal-Zellerfeld 38678, Germany.
Fax: +49 5323 72 3570.
E-mail address: strube@itv.tu-clausthal.de (J. Strube).

http://dx.doi.org/10.1016/j.reffit.2017.03.001

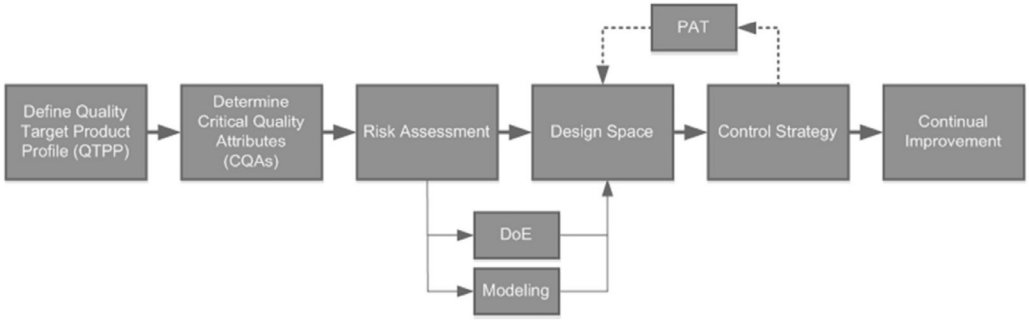

Fig. 1. The QbD-approach to quality assurance.

(QbD)-approach to process development for biologics. The QbD-approach in its current form is shown in Fig. 1.

The implementation of QbD enables risk focused cooperation between competent agencies and manufacturers and offers a higher degree of process robustness and flexibility (see Fig. 2). Plants have in general a broad variety of compounds depending on growth and harvesting conditions. A suitable process design rated by the QbD principals ensures a robust process from extraction to purification even if the feedstock has a great variety. Over the last few years, a number of exemplary case studies have been published regarding different types of products [10–12].

Also first commercial applications containing QbD-elements have been assessed and approved [13,14]. QbD was originally intended by authorities for biologics, where product quality proven completely by analytics is not desired. Product quality has to be controlled by means of appropriate processes and operations as well.

These demands were developed in order to improve patients' safety by optimal drug quality at more efficient manufacturing processes reducing costs for healthcare systems. Furthermore, production of biologics includes feedstock variability and complex multistep manufacturing processes in batch operation with variable lots – condition, which apply to botanicals as well.

Transferring the QbD-approach to botanicals the natural variability of plant material needs to be addressed, establishes a quality standard and offers increased process knowledge, three aspects with very high significance for herbal products [15].

The focus on quality is important for phytopharmaceuticals, not only for the safety of patients, but to promote standardization and progress regarding the definition of quality of plant based products.

2. Example process

The spectrum of herbal products ranges from edible plants, general extracts to highly purified components, depending on the number and type of unit operation [16]. To cover the complete spectrum of products, the production of 10-deacetylbaccatin III (10-DAB) was chosen to showcase the methodical approach to process development including QbD-elements.

The development process consists of a series of steps, shown in Fig. 3.

The QbD approach relies on solid process comprehension in order to control product quality throughout the process. Especially, the implementation of rigorous process models provides an effective method to establish the required process knowledge. They ensure comparability between process scales within rigorous models, which is why they represent the missing link, connecting process risk assessment and process characterization [17–19].

A key concern regarding herbal products is the definition of quality. The QbD-approach demands a definition of quality coherent with the intended therapeutic application of the medication. The **"quality target product profile" (QTTP)** is based on pharmacodynamics and pharmacokinetic studies, as well as toxicological studies, and usability. Based on the QTTP, relevant attributes of the product need to be identified.

The key quality attribute for the extraction of 10-DAB from yew is the purity of the target component. The purity is defined as the amount and number of other components with similar elution properties during the final separation in relation to the target component, as established in a previous work [20].

The example process for obtaining 10-DAB from yew, material and methods as well as analytics were proposed by Ref. [20]. A general flow sheet is shown in Fig. 4.

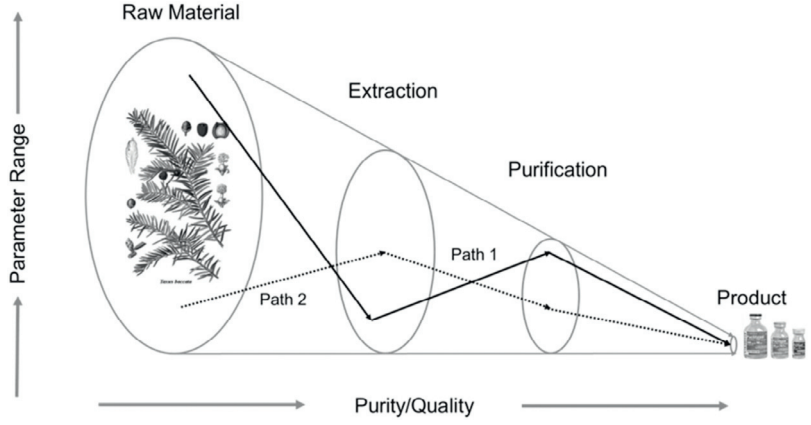

Fig. 2. Inside design space boundaries.

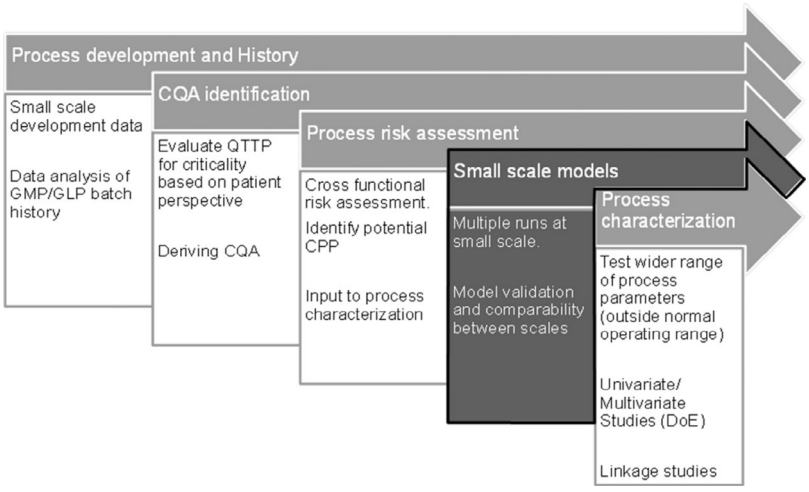

Fig. 3. Process development including QbD.

- Prior to extraction, the yew twigs and needles are shredded and sieved (not shown in the flowsheet).
- After extraction with a mixture of acetone and water.
- The acetone is recycled, resulting in the precipitation of chlorophyll.
- 10-DAB is then purified from the aqueous concentrate by a one-stepped liquid–liquid extraction with ethyl acetate which is sufficient to capture and purify most of the target component.
- Finally, 10-DAB is isolated and further purified by batch chromatography utilizing reverse phase chromatography using acetonitrile and water as eluents.

In the following, the different QbD-elements are demonstrated along the processing of the yew material:

1. The **risks** of the preparation of plant materials are assessed.
2. Based on this risk assessment, the **impact factors** on extraction are evaluated.
3. The approach to **process control** is shown by deriving the design space for the final separation step and pointing out the linkage to prior unit operations.

2.1. Risk assessment of plant material preparation

Risks regarding raw material are a main source of viability for botanicals. The environment during growths and harvesting conditions can result in very different compositions of active substances. The concentration of 10-DAB has been shown to vary greatly between different parts and populations of yew trees [21].

The design of a process according to QbD-principles aims to understand the implications of variability for the product quality. The first step is the preparation of yew needles for solid–liquid extraction. The needles are shredded and sieved to allow a fast and efficient extraction.

An exemplary, early risk assessment for the preparation procedure prior to solid–liquid extraction is shown in Fig. 5. The

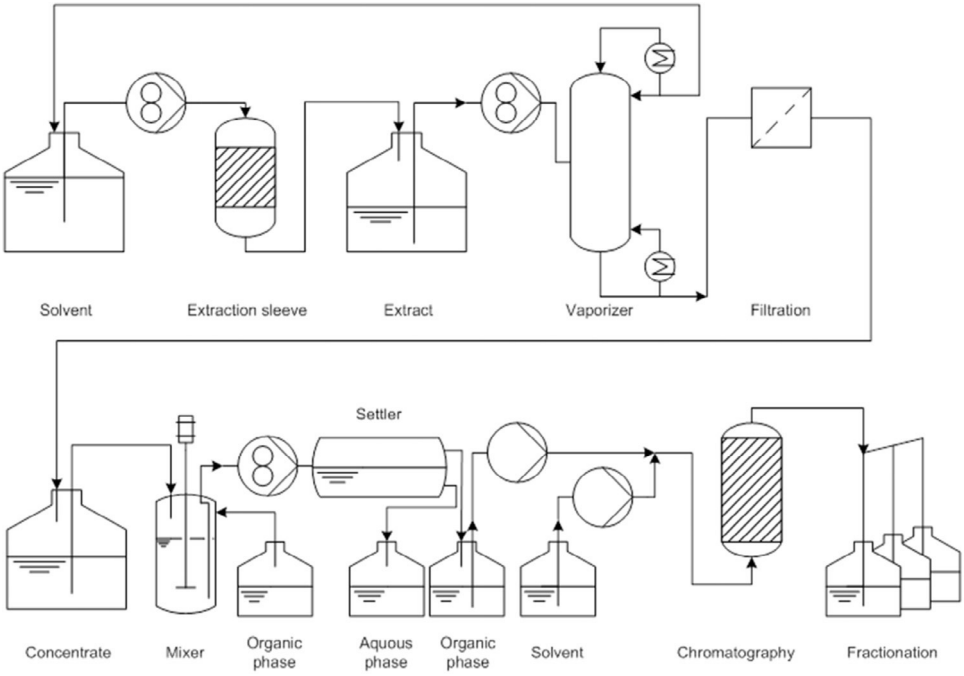

Fig. 4. 10-DAB production process.

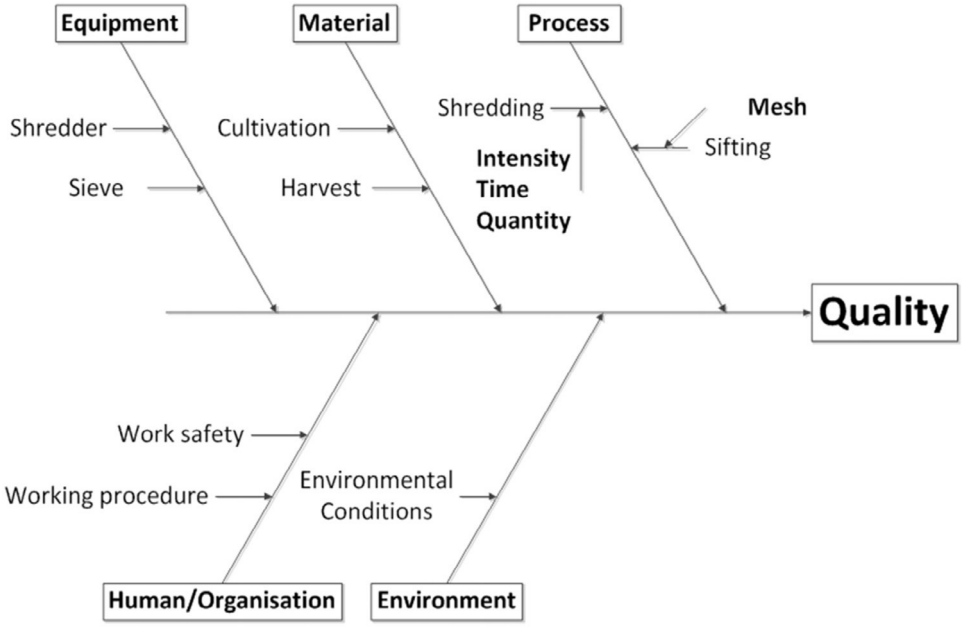

Fig. 5. Breakdown of preparation procedure.

Ishikawa-method is based on the cause and effect relation between quality and risk factors and can be used to get a general overview [22]. The Ishikawa-diagram is quick and easy to understand, which makes it ideal for efficient risk assessment during early development phases.

Every revealed risk should be addressed during process development. The influence on quality of human error, equipment and environment can be mitigated by means of efficient management, by enforcing standard operation procedures or maintenance schedules. Risks regarding process and materials, in this case shredding and sifting, are the most relevant for process characterization.

With increasing process comprehension, the valuation of risks can change during the development. Therefore, the assessment process should be reviewed over time, to ensure a conclusive risk evaluation. It is important to note that risk assessment should not exclude risks based on presumptions. Exclusion of risks without proper, scientifically sound evaluation compromises overall process robustness. All parameters must be considered until valid data are generated to rule out their impact on quality.

A comprehensive risk assessment is the basis for subsequent process characterization. A popular method with a higher degree of detail categorizes risks based on occurrence, impact and detectability of the respective risk. The so called "**failure mode and effects analysis" (FMEA)** can give additional information which is useful during process characterization.

The qualitative FMEA shown in Fig. 6 is based on the Ishikawa-diagram for extraction, similar to the one shown in Fig. 5. The ranking of parameters according to occurrence, impact and detectability is used to document the risk discussion process and improve.

Ranking risks according to the "risk priority number" (RPN) can give an additional level of detail. The RPN is defined as the product of an impact, occurrence and detectability factor, ranging from 1 to 10, resulting in a number between 1 and 1000 [23]. This kind of quantitative risk ranking may lead to underestimation of low scoring factors, as long as the risk factors are chosen presumptively.

2.2. Impact factors of preparation on extraction of yew material

On the basis of the comprehensive risk assessment described before, the identified factors are investigated and ranked regarding the amount of impact on the quality of the product. One of the most efficient ways to determine the impact of parameters on quality are **experimental designs based on statistical methods** (design of experiments, DoE).

As shown in Fig. 5 the main process steps for preparation are shredding and sieving of yew twigs. According to the FMEA, relationships between particle size, solvent temperature, flow rate and water content of plant material have been investigated using a semi factorial DoE-approach including center point. The experimental design consists of eight experiments and a triplicate center point, resulting in a total of 11 experiments.

The semi factorial DoE can be used to evaluate risks by showing interactions between factors and allows identification of factors, which impact the desired quality. Fig. 7 shows the impact of main factors, with water content and particle size showing the highest impact. Based on these impact factors, the most robust process can be derived.

Factors with negligible impact on quality can be set to the most convenient value inside the investigated ranges. The remaining factors should be ranked based on their controllability and measurability, which Fig. 8 exemplifies. Process robustness can be maximized by adjusting those factors to allow for more variability regarding the more challenging parameters:

- First, flow rate can be precisely controlled technically.
- Second process temperature can be adjusted and measured precisely.
- Water content of the solvent can be specified prior to extraction and adjusted during mixing. These process parameters can be classified as well controlled.
- Particle size depends on plant morphology and lignification. These factors are more challenging to control, making a wider acceptable range desirable for high process robustness. Therefore, the risk priority of particle size and water content is adjusted.

2.3. Control strategy for separation of 10-DAB from yew extract

Following risk assessment and risk ranking, a **control strategy** has to be designed to guarantee the quality requirements of the product. In order to control a process, the interactions between

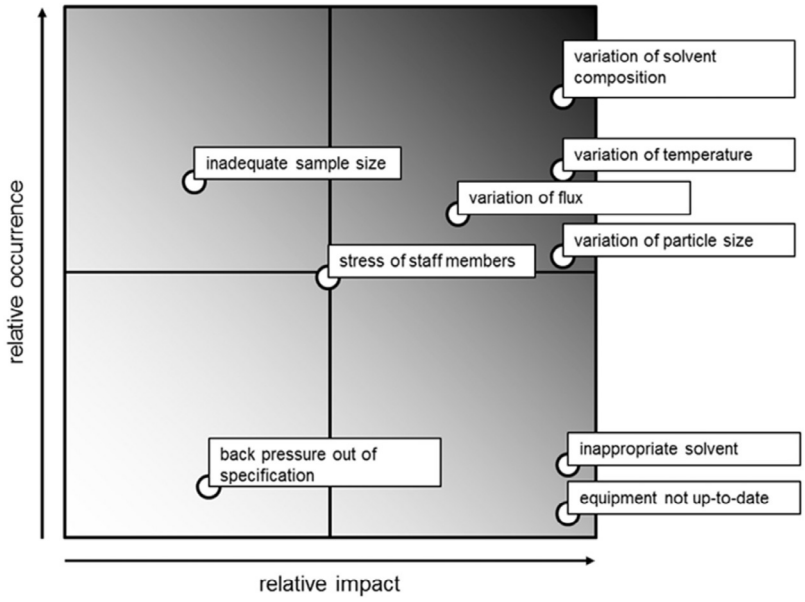

Fig. 6. Qualitative FMEA approach to risk assessment.

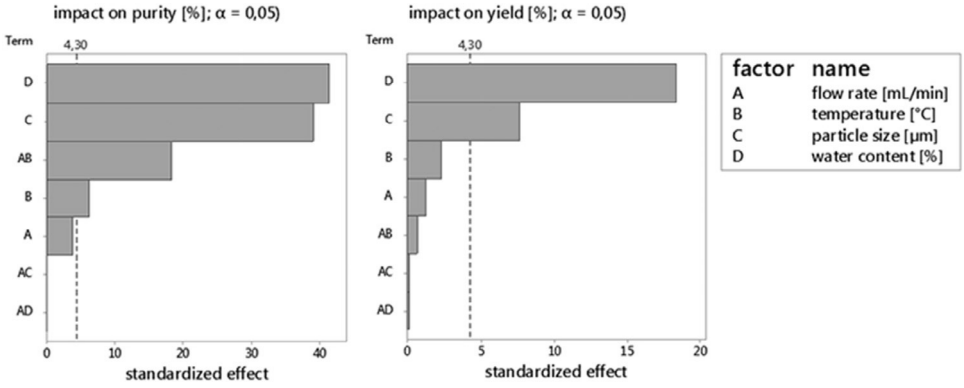

Fig. 7. Impact factors on yield on purity of yew extract.

Fig. 8. Risk ranking to ensuring process robustness.

process parameters and quality attribute have to be known inside the characterized space.

An expanded experimental design, based on the experimental studies during risk evaluation and ranking, is an efficient way to increase the availability of information. The resulting space, which is controlled by this strategy, is usually referred to as **"design space"** (see Fig. 9).

The application of previously introduced methods, i.e. risk assessment and sensitivity studies, has shown that separation may be influenced by variability in flow-rate, load, solvent composition and adsorbent properties among others [19]. According to QbD-principles, a suitable solvent composition and adsorbent should be chosen based on scientific insight and proven product knowledge. Screening of different phases and solvents was done using thin layer chromatography to determine suitable chromatographic conditions [24]. The best separation could be achieved on adsorbent with nonpolar properties.

Solvent screening for modeling and optimization has shown suitable solubility in solvents like acetone and ethanol [20].

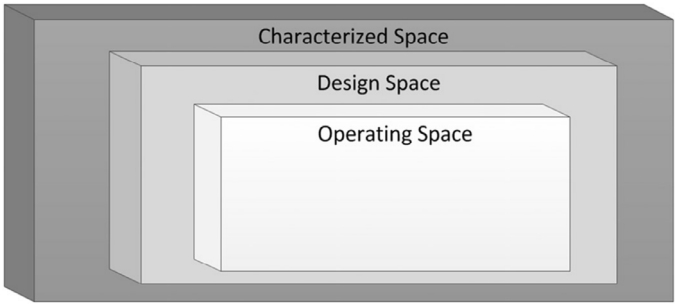

Fig. 9. Relation of tested space, design space and operation space.

The influences of flow-rate and load on the separation are studied in semi-preparative scale (see Fig. 10). The method of separation is scaled up from analytic scale using the linear scale-up approach.

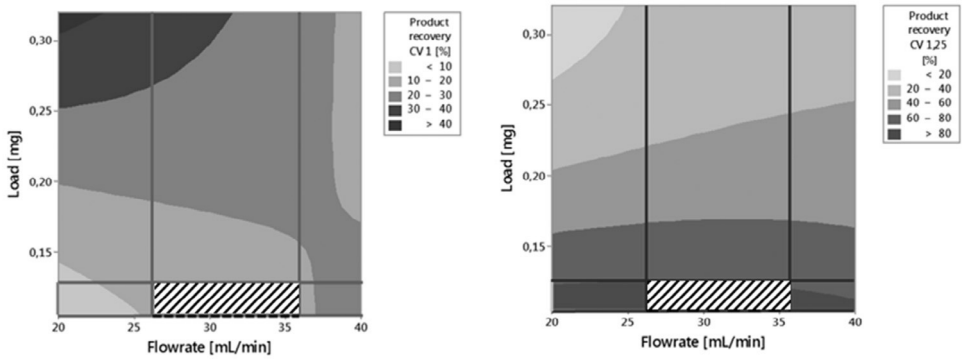

Fig. 10. Design space of the chromatographic separation of 10-DAB.

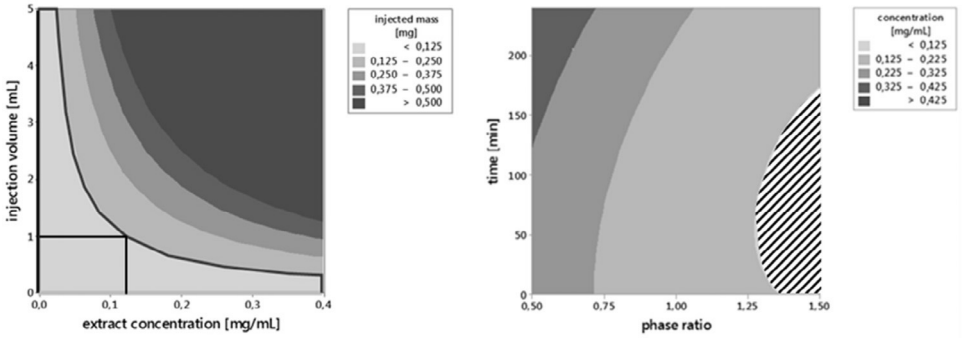

Fig. 11. Risk linkage between column load and extraction phase ratio.

The assurance of **process robustness** is a main objective during QbD-based process development. Multivariate parameter studies can be used to evaluate significant risks, by increasing process knowledge regarding the risk interaction.

The main proportion of product is recovered between 1.25 and 1.5 column volumes (CV) after injection. The contour-diagram can be derived from regression models based on the experimental design. The highlighted areas in Fig. 10 show the highest recovery rate for all test configurations between 0.1 to 0.3 mg injected target component and 20–40 mL/min flow rate. The limitation of 0.125 mg load has implications on the preceding process steps. Column load is determined by the concentration of extract after liquid–liquid extraction and the volume of injection. This connection is shown in Fig. 11.

Inside the highlighted area, the injected amount of product will be smaller 0.125 mg, meeting the requirements of the chromatography design space.

3. Conclusion

The **concept of risk**, including risk assessment and control are essential to the QbD-based development and offers the possibility to develop efficient and robust processes coping with natural variability of feedstocks in complex manufacturing procedures needing manifold analytics in QA, only based on sound scientific data.

The investigation of process parameters outside the normal operation range, including the implication for subsequent process steps, increases the flexibility during operation and results in a higher degree of overall process robustness and efficiency. Efficiency generates sustainability dealing with natural resources and economy of healthcare systems under **societal needs** at improved patients' safety.

The isolation of **10-DAB from yew** can be used as an example process to showcase risk assessment and risk evaluation for different types of operations.

The **risks** during preparation of plant material and extraction are shown, utilizing basic risk assessment tools. Risk impact is evaluated by the example of solid–liquid extraction. **Impact factors** are generated using DoE, which can be used to minimize experimental effort and time during development and ensure statistical significance of the data. In this study a semi-factorial design was used because it is sufficient to screen for major effects. A maximum of process robustness is ensured by ranking impact factors according to controllability and measurability. For the extraction of natural components from plants the natural variety of the plant material plays an important role toward the total process. Nevertheless solid–liquid extraction is not capable to ensure the purity of the product because of its relatively small selectivity. Hence this unit operation has to be optimized to maximum yield in order to use the natural resources in an efficient way. Product purity and therefore consumer security are assured by chromatography. The **control strategy** of the chromatographic separation including the risk linkage of column load to the phase ratio during liquid–liquid extraction demonstrates the design space concept.

The QbD-approach is on the way to become the new standard in process development for biological pharmaceuticals. With the approval of the first applications containing the QbD-approach for process development, a milestone has been reached.

The ICH-guideline Q8 describes QbD as an enhanced approach to process development. The QbD-concept should not be seen as a revolutionary approach to process design, but as an extension [25]. While additional effort during development is minimal for the enhanced approach to process development, the gains regarding process comprehension are clearly significant. Furthermore, the QbD-approach harmonizes the language throughout industry and involved agencies, reducing communication difficulties and consequently improving the application process.

The focus on risk based process development synergizes with initiatives by the agencies promoting continuous processing and

rigorous physico-chemical modeling. The focus on data driven decision-making supports the use of physico-chemical modeling for additional clarity, regarding possible interactions between risks and process parameters. Simultaneously, the integration of **predictive process modeling** significantly reduces experimental expenses, time and environmental impact of process development gaining sound quantitative process comprehension. The establishment of validated small scale models bridges the gap between risk assessment and process characterization.

The QbD-approach should not be seen as a burden imposed by regulatory agencies but as a chance to integrate a higher degree of flexibility into process operation, in order to adapt reliably to processes with natural variability. The advances in process analytical technologies (PAT), for example in the field of spectroscopy, improve the possibilities to measure and control continuous processes. The combination of PAT and QbD is justifiably envisioned by the regulatory agencies as the future of the pharmaceutical production process. The application of QbD provides:

- Robust and reliable processes, assuring product quality.
- Solid process understanding, granting more flexibility
- Support for innovative methods, encouraging rigorous models.
- Economic development of processes, reducing cost.
- Risk focused development, supporting communication of regulatory and industry

Besides the administrative definitions and lengthy explanations of the guidelines, the intellectual short-cut of the approach could be summed up as “continuous Good Science Practice (cGSP)” in order to be able to file data-driven decisions. Applying QbD-methods combined with rigorous process modeling it could be exemplified that QbD is more chance than obstacle for manufacturers.

References

[1] F. Carmona, S. Pereira, Herbal medicines: old and new concepts, truths and misunderstandings, Rev. Bras. Farmacogn 23 (2013) 379–385.
[2] M. Saxena, J. Saxena, R. Nema, D. Singh, A. Gubpta, Phytochemistry of medicinal plants, J. Pharmacog. Phytochem 1 (2015) 1–182.
[3] G. Cravotto, L. Boffa, L. Genzini, D. Garella, Phytotherapeutics: an evaluation of the potential of 1000 plants, J. Clin. Pharm. Ther 35 (2010) 11–48.
[4] T.-T. He, C.O.L. Ung, H. Hu, Y.-T. Wang, Good manufacturing practice (GMP) regulation of herbal medicine in comparative research: China GMP, cGMP, WHO-GMP, PIC/S and EU-GMP, Eur. J. Integr. Med 7 (2015) 55–66.
[5] B.H. Kroes, The legal framework governing the quality of (traditional) herbal medicinal products in the European Union, J. Ethnopharmacol 158 (2014) 449–453.
[6] J. Wiesner, W. Knöss, Future visions for traditional and herbal medicinal products – a global practice for evaluation and regulation? J. Ethnopharmacol 158 (2014) 516–518.
[7] A. Burton, M. Smith, T. Falkenberg, Building WHO’s global strategy for traditional medicine, Eur. J. Integr. Med 7 (2015) 13–15.
[8] T. Sikora, Good manufacturing practice (GMP) in the production of dietary supplements, in: Dietary Supplements, Elsevier, 2015, pp. 25–36.
[9] Food and Drug Administration (FDA), Pharmaceutical cGMP for the 21st century: a risk based approach, 2004.
[10] CMC Biotech Working Group (CMC-BWG), A Mab: a case study in bioprocess development: product development and realisation case study: a Mab, 2009.
[11] CMC Vaccines Working Group (CMC-VWG), A-VAX applying Quality by Design to vaccines, 2012.
[12] Ministry of Health, Labour and Welfare (MHLW), Sakura case study: establishing design space in critical steps and control strategy. Quality overall summary mock P2 (description examples), 2009.
[13] European Medicines Agency (EMA), CHMP assessment report Perjeta, 2013.
[14] European Medicines Agency (EMA), CHMP assessment report Gazyvaro, 2014.
[15] I.A. Khan, T. Smillie, Implementing a “Quality by Design” approach to assure the safety and integrity of botanical dietary supplements, J. Nat. Prod 75 (2012) 1665–1673.
[16] R.A. Aziz, R. Sarmidi, S. Kumaresan, Z.M. Taher, F. Cwan Yee, Phytochemical processing: the next emerging field in chemical engineering – aspects and opportunities, J. Kejuruteraan Kimia 3 (2004) 1–13.
[17] S. Both, J. Eggersglüß, A. Lehnberger, T. Schulz, T. Schulze, J. Strube, Optimizing established processes like sugar extraction from sugar beets – design of experiments versus physicochemical modeling, Chem. Eng. Technol 36 (2013) 2125–2136.
[18] M. Kassing, F. Svec, U. Jenelten, J. Schenk, R. Hänsch, J. Strube, Combination of rigorous and statistical modeling for process development of plant-based extractions based on mass balances and biological aspects, Chem. Eng. Technol 35 (2012) 109–132.
[19] C. Helling, J. Strube, Modeling and experimental model parameter determination with Quality by Design for bioprocesses, in: Biopharmaceutical Production Technology, Wiley-VCH-Verl, Weinheim, 2012, pp. 409–445.
[20] M. Sixt, I. Koudous, J. Strube, Process design for integration of extraction, purification and formulation with alternative solvent concepts, C. R. Chim 19 (2016) 733–748.
[21] R. Kelsey, N. Vance, Taxol and cephalomannine concentrations in the foilage and bark of shade-grown and sun-exposed Taxus brevifolia trees, J. Nat. Prod 55 (7) (1992) 912–917.
[22] K. Ishikawa, Introduction to Quality Control, 3A Corp., Tokyo, 1990.
[23] German Commission for Electrical, Electronic and Information Technologies of DIN and VDE, Failure mode and effects analysis, 2015.
[24] L. Uhlenbrock, Quality by Design als Werkzeug der Qualitätssicherung bei der Extrakton pflanzlicher Arzneistoffe am Beispiel von Eibe Master thesis, 2017.
[25] ICH Expert Working Group (ICH EWG), Pharmaceutical development Q8(R2): ICH harmonised tripartite guideline Q8(R2), 2009.